

Genetic and Morphological Variations within *Laudakia microlepis* (Blanford, 1874) (Sauria: Agamidae) Populations in Southeastern Iran with Description of a New Subspecies

Nasser SANCHOOLI^{1,2}, Hassan RAHIMIAN^{1*}, Nasrullah RASTEGAR-POUYANI^{3,4} and Eskandar RASTEGAR-POUYANI⁵

¹ Faculty of Biological Sciences, College of Science, University of Tehran, Tehran, Iran

² Department of Biology, Faculty of Science, Zabol University, Zabol, Iran

³ Department of Biology, Faculty of Science, Razi University, Kermanshah, Iran

⁴ Iranian Plateau Herpetology Research Group (IPHRG), Razi University, Kermanshah 6714967346, Iran

⁵ Department of Biology, Faculty of Science, Hakim Sabzevari University, Sabzevar, Iran

Abstract A new subspecies of *Laudakia microlepis* (Blanford, 1874) is described, from Taftan Mountain in southeastern Iran, based on morphological and genetic characteristics. This new subspecies is distinguished from the nominal subspecies by having a yellowish head along with the following combination of characters: a relatively larger body size; different number of scales around middle of body; larger, weakly keeled, dorsal median scales arranged in 11–13 oblique longitudinal rows; 123–136 scales in a single row from posterior edge of gular fold to vent; 27–31 scales in the first complete whorl around the tail. The type locality of *Laudakia microlepis taftanica* ssp. nov., is hereby, reported as the Taftan Mountain, about 300 kilometer southeast of the nominal subspecies known range.

Keywords *Laudakia microlepis taftanica* ssp. nov., taxonomic status, ND4 gene, Sistan and Baluchistan province

1. Introduction

The genus *Laudakia* comprises about 20 species, mainly occurring in highland and mountainous regions of the central and southern Asia. Of those 20 species, at least five have been reported from Iran (Šmid *et al.*, 2014). Using morphology-based, non-phylogenetic analysis of *Laudakia* species, Baig *et al.* (2012) divided the genus into three genera; *Stellagama*, *Paralaudakia* and *Laudakia*. But, shortly after that, a robust molecular phylogenetic analysis strongly supported monophyletic status of the genus *Laudakia*, suggesting that the changes were not supported (Pyron *et al.*, 2013).

Laudakia microlepis was first described by Blanford (1874) from two localities in Iran: Khane-Sorkh Pass,

with elevation of about 2 750 m, between Sirjan and Kerman, southeastern Iran, and Kushk-e-Zard in south-central Iran. Subsequent studies, however, determined that further to southern Iran, *L. microlepis* is distributed in southern and western Pakistan, Afghanistan, and parts of Turkmenistan (Baig *et al.*, 2012). The taxonomic status of *L. microlepis* has been controversial ever since its description. For instance, while Blanford (1876) pointed to the very close relationships between *L. caucasia* and *L. microlepis*. Wetstein (1953) considered *L. microlepis* as a subspecies of *L. caucasia*, Rastegar-Pouyani and Nilson (2002) and Baig *et al.* (2012) considered the two taxa as distinct species with sometimes sympatric distributions.

In the present communication, we describe a new subspecies of *Laudakia microlepis* based on comparison between distinguishing morphological characteristics and by comparing mitochondrial ND4 gene sequences, among specimens collected from Taftan Mountain with those from the type locality of *L. microlepis*.

* Corresponding author: Dr. Hassan RAHIMIAN, from College of Science, University of Tehran, Tehran, Iran, with his research focusing on lizards of Iran.

E-mail: h.rahimian@ut.ac.ir

Received: 26 November 2014 Accepted: 29 May 2015

2. Material and Methods

Fourteen specimens were collected in August 2011, from Taftan Mountain ($28^{\circ} 36' 36.6''$ N $61^{\circ} 04' 51.1''$ E, elevation 2 530 m), in Sistan and Baluchistan province, southeastern Iran (Figure 1). In addition, five specimens were collected from Khane Sorkh, between Sirjan and Kerman. Specimens are deposited in the Zoology Museum of the University of Tehran (ZUTC).

Morphology Specimens collected from the Taftan Mountain, comprising eight males and six females, were preserved in 70% ethanol and deposited in the Zoology Museum of University of Tehran (ZUTC). All measurements are carried, to the nearest 0.01 millimeter, using vernier calipers, and scales on different parts of the body were counted with aid of a stereomicroscope. The following characteristics were considered: Scales around mid-body (SQ), number of scales in a single row around the widest part of body; Large Vertebral Scales (LVS), number of longitudinal rows of enlarged scales on the vertebral region; Anus-Gular Fold Scales (AGFS), number of ventral scales in a single row from posterior edge of gular fold to the vent; Fifth Caudal Whorl Scales (FCWS) scales around the fifth caudal whorl just behind the vent; Snout-Vent Length (SVL) from tip of the snout to the anterior edge of cloaca; Tail Length (TL) from posterior edge of cloaca to the tip of the tail; Head Length (HL) from tip of the snout to the posterior edge of tympanum; Head Width (HW) measuring widest part of the head; Head Height (HH) from top of the head to the lower jaw. Data were analyzed using statistical software SPSS (version 22). The significance level for all tests were set at $P < 0.05$. Descriptive statistics were carried out to explore the means, standard errors and ranges of the characters.

Averages of those traits for *L. caucasia*, and *L. erythrogaster* are adopted from Rastegar-Pouyani and Nilson (2002).

Molecular analyses Fourteen specimens belonging to the genus *Laudakia* (for details refer to Table 1) were used in the molecular analyses. DNA was extracted using non-organic DNA Extraction Procedure (Proteinase K and Salting out). MT-ND4 (NADH dehydrogenase subunit 4; from 1 to 642, ND4; from 643 to 801, tRNA His [complete], tRNA Ser [complete] and tRNA Leu [Partial]) was amplified using standard PCR procedures with the following primers: ND4F, 5'-CACCTATGA CTACCAAAAGCTCATGTAGAAGC-3' (Thaung *et al.*, 2009) and LeuR, 5'-CATTACTTTACTTGGAT TTGCACCA-3' (Arevalo *et al.*, 1994). PCR reactions

performed in 20 μ l with the following conditions: Initial denaturation stage of 95°C (05:00) followed by 36 cycles with denaturation at 95°C (00:40), annealing at 50°C (00:40) and extension at 72°C (01:40) then single extension cycle at 72°C (05:00). The PCR products were sequenced with an automatic DNA sequencer using manufacture's protocols by BIONEER (Daejeon, Republic of Korea). In order to conduct a more accurate molecular analysis, four sequences belonging to *Laudakia lehmanni* (KF691670.1), *L. microlepis* (KF691699.1), *L. stoliczkanai* (AF128519.1) and *L. caucasia* (AY053998.1) were retrieved from GenBank (<http://www.ncbi.nlm.nih.gov>) and included to our dataset. Nucleotide sequences were aligned using ClustalW, as implemented in Bioedit version 7.0.5.3 (Hall, 1999) with default parameters. Using the software package Mega 6 (Tamura *et al.*, 2013) genetic distances between taxa were calculated. Bayesian analysis was carried out using Mr. Bayes 3.1.2 (Huelsenbeck and Ronquist, 2001). Bayesian analysis was performed with two runs and four chains for each run for 4 million generations and the current tree was saved every 100 generations. A 50% majority rule consensus tree was produced from the posterior distribution of the trees, and the posterior probabilities were calculated as the percentage of samples recovering any particular clade, with posterior probabilities $P \geq 95\%$ indicating significant support.

3. Results

A data set of 677bp of ND4 following by tRNAs, from 677 to 844, including tRNA His (complete) + tRNA Ser (complete) + tRNA Leu (Partial) was generated. Genetic divergence (Kimura 2 parameters) among different specimens varied from 5.5 to 17.8% (Table 2). Phylogenetic relationships (BI inference) between *Laudakia microlepis* and Taftan specimens based on 844 nucleotides clearly indicate that Taftan specimens fall apart from *L. microlepis*, as illustrated in Figure 2.

Based on the results, presented here, the *Laudakia microlepis* populations are distributed on the southeastern parts of Iran and at least some of them, namely that of the Taftan Mountain, are genetically different from other populations of *L. microlepis*, sufficient to be considered as a subspecies of the nominal species. As such we call this new subspecies, *Laudakia microlepis taftanica* ssp. nov., against the nominal species *L. m. microlepis*.

3.1 Description *Laudakia microlepis taftanica* ssp. nov. (Figure 3 A, B and C)

Holotype and type locality An adult male (Figure 3A,

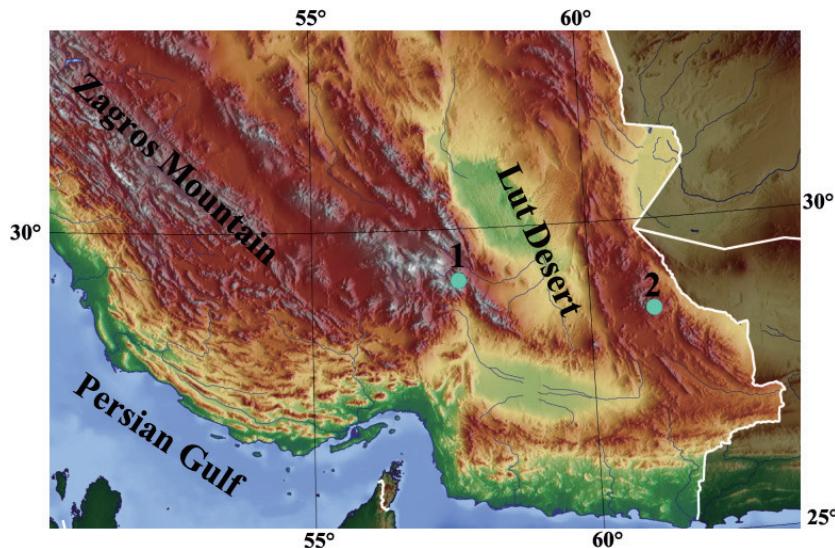


Figure 1 Topographic map viewing the southern part of Iran showing the type locality of the *Laudakia microlepis* (Khane Sorkh) in Kerman Province (1) and location of Taftan Mountain on the southeastern corner of Iran Plateau (2).

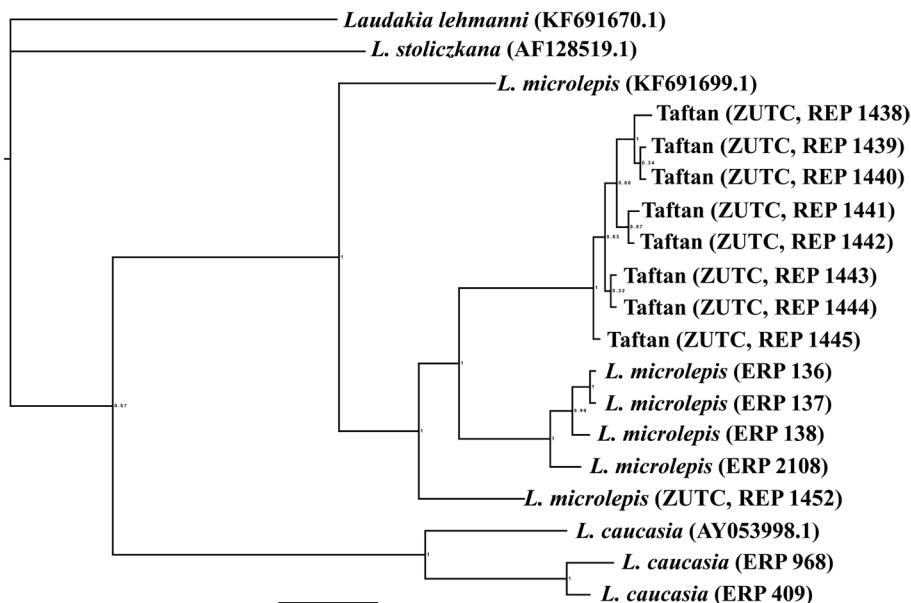


Figure 2 Phylogenetic relationships (BI inference) among *Laudakia microlepis* and Taftan specimens (Shown as Taftan) based on 844bp ND4 sequences.

B), ZUTC, REP 1438, collected on 25th August 2011, 25km north of Khash, Sistan and Baluchistan province, Southeastern Iran (28° 36.0' 25.1" N 61° 04' 47.3" E).

Paratypes Thirteen specimens (seven males and six females) collected, from the same locality as holotype (see Appendix).

Diagnosis Body size large, head and body moderately depressed; grayish-black dorsally; a cutaneous fold on each side of the neck; a dorsolateral cutaneous fold on each side of the body with smooth scales; enlarged vertebral scales, weakly keeled; body scales small; no

patch of enlarged mucronate scales on flanks; tail divided into two distinct segments, each composed of two whorls of scales.

Description of Holotype Upper head scales heterogeneous, spiny scales around the tympanum relatively smaller than scales around orbit, spiny scales on each side of the neck; enlarged mid-dorsal scales in 13 rows, median dorsal scales equal in size with ventral scales; ventral scales smooth, scales on upper limbs larger than median dorsal scales and distinctly keeled; dorsal scales heterogeneous, scales on flanks smaller than



Figure 3 *Laudakia microlepis taftanica* ssp. nov.: (A) Preserved specimen lateral view; (B) Preserved specimen dorsal view; (C) Live specimen in its natural habitat; and (D) Natural habitat of *Laudakia microlepis taftanica* ssp. nov., about 30 km north of Khash, Sistan and Baluchistan Province, eastern Iran.

mid-dorsal scales; gular scales smooth with patches of yellowish scales; 198 scales around middle of the body, 110 scales in single row from gular fold to anterior edge of vent; 27 scales around fifth caudal whorl just behind vent; with mid-ventral and pre-anal callous scales.

Coloration and color pattern Dorsal and ventral surfaces of the body proper grayish-black; posterior part of the head, around tympanum and dorsal part of the neck yellowish (Figure 3A, B); a patch of yellowish scales on the anterior part of the head; proximal part of the tail with black-cream rings, distal part of tail black.

Measurements (in millimeters) Total length 276.6; SVL 121.5; TL 155.1; HL 31.8; HW 23.2; HH 15.8; SN 6.4; NN 8.4; FLL 69.0; HLL 102.3; GFV 81.4; MTrW 41.4; MTaW 18.7.

Variations of type series All paratypes closely resemble the holotype in both morphometric and meristic traits. However, there are some minute differences between male and female paratypes. The folds on sides of neck are more developed in males than those in females. In some females mid-ventral and pre-anal callous scales are absent. In males, scales of upper side of limbs are more

strongly keeled than those in females. In females mid-dorsal scales are larger than ventral scales. Furthermore, in some females, dorsal surface contains scattered pale-green or brownish scales. In some males, except for black rostrum, other parts of the head are yellow in coloration.

Male paratypes All male paratype specimens ($n = 7$) were similar to the holotype in both morphometric and meristic characteristics, with slight variations in some specimens. Range and mean (in parenthesis) of different characteristics are as follows: The SVL 101.6–129.6 (119.7), TL 127.8–172.1 (162.8), HL 26.8–33.0 (29.9), HW 20.1–25.4 (23.7), HH 10.8–16.1 (14.5), SN 4.7–6.4 (5.3), NN 6.5–7.8 (7.3), FLL 53.7–79.2 (67.4), HLL 76.6–106.5 (100.5), GFV 10.9–108.7 (83.2), MTrW 30.6–44.0 (39.1), MTaW 13.9–19.9 (18.2), SQ 184–197 (191.8), LVS 11–13 (11.6), AGFS 120–136 (127.3), FCWS 26–31 (28.5). Mid-dorsal scales enlarged, almost homogeneous, weakly keeled; all dorsolateral, gular and ventral scales similar to the holotype; coloration almost identical to that of the holotype.

Female Paratypes Females ($n = 6$) rather different from the holotype. Range and mean (in parenthesis) of different

Table 1 List of the specimens studied in the present communication and their localities. ERP: personal collection of Dr. Eskandar Rastegar Pouyani, NCBI: National Center for Biotechnology Information ZUTC: Zoology Museum of University of Tehran.

Species	Museum Number	NCBI Accession Number	Geographic coordinates of sampling location	Elevation (m)	Locality
<i>L. caucasia</i>	ERP 409	Pending	34° 33' 56"N 60° 07' 18"E	1036	Khorasan Razavi Province, Khaf
	ERP 968	Pending	35° 16' 17"N 57° 59' 39"E	1031	Khorasan Razavi Province, Bardsan
<i>L. microlepis microlepis</i>	ERP 2108	Pending	29° 49' 08"N 56° 04' 05"E	2750	Kerman Province, Khane-Sorkh Pass
	ERP 138	Pending	29° 05' 50"N 57° 34' 14"E	3150	Kerman Province, North of Jiroft
	ERP 136	Pending	29° 05' 57"N 57° 34' 10"E	3141	Kerman Province, North of Jiroft
	ERP 137	Pending	29° 05' 51"N 57° 34' 16"E	3148	Kerman Province, North of Jiroft
	ZUTC (REP 1438)	Pending	28° 36' 25"N 61° 04' 47"E	2530	Sistan and Baluchistan Province, Taftan Mountain
<i>L. microlepis taftanica</i>	ZUTC (REP 1439)	Pending	28° 36' 20"N 61° 04' 47"E	2521	Sistan and Baluchistan Province, Taftan Mountain
	ZUTC (REP 1440)	Pending	28° 36' 21"N 61° 04' 49"E	2534	Sistan and Baluchistan Province, Taftan Mountain
	ZUTC (REP 1441)	Pending	28° 36' 32"N 61° 04' 39"E	2518	Sistan and Baluchistan Province, Taftan Mountain
	ZUTC (REP 1442)	Pending	28° 36' 25"N 61° 04' 40"E	2524	Sistan and Baluchistan Province, Taftan Mountain
	ZUTC (REP 1443)	Pending	28° 36' 26"N 61° 04' 40"E	2533	Sistan and Baluchistan Province, Taftan Mountain
	ZUTC (REP 1444)	Pending	28° 36' 25"N 61° 04' 44"E	2526	Sistan and Baluchistan Province, Taftan Mountain
	ZUTC (REP 1445)	Pending	28° 36' 27"N 61° 04' 30"E	2519	Sistan and Baluchistan Province, Taftan Mountain

Table 2 Genetic distances (Kimura 2 parameters) between *Laudakia microlepis*, *L. microlepis taftanica* ssp. nov., and related taxa.

	1	2	3	4	5
<i>Laudakia lehmanni</i>					
<i>Laudakia stoliczkanai</i>	0.145				
<i>Laudakia caucasia</i>	0.156	0.178			
<i>Laudakia microlepis</i> (unknown locality)	0.159	0.154	0.162		
<i>Laudakia microlepis</i> (Near Type-locality)	0.17	0.163	0.159	0.065	
<i>Laudakia microlepis taftanica</i>	0.172	0.172	0.16	0.078	0.055

characteristics are as follows: The SVL 99.7–119.8 (114.2), TL 121.9–162.8 (146.6), HL 22.7–29.3 (26.4), HW 19.8–23.7 (21.6), HH 11.4–14.1 (13.1), SN 3.6–5.7 (4.5), NN 6.0–7.2 (6.5), FLL 52.4–66.1 (59.4), HLL 75.6–99.9 (84.1), GFV 80.7–108.2 (88.7), MTrW 30.0–47.8 (40.3), MTaW 12.1–16.7 (14.8), SQ 182–200 (192), LVS 10–13 (11.3), AGFS 118–130 (123.5), FCWS 23–33 (27.2). Mid-dorsal scales almost homogeneous, weakly keeled; dorsolateral scales smaller than median dorsal scales; mid-ventral and pre-anal callous scales present in only one specimen; females coloration different from that of the male holotype in the presence of scattered pale-green scales on posterior dorsal and hind limbs as well as patches of yellow scales on shoulders.

4. Discussion

In comparison between different traits in specimens of *Laudakia microlepis* collected from Taftan Mountain and

those collected from the type locality, we found some variations that, in combination with genetic variations, could justify the erection of a subspecies. Some of those traits have been used to distinguish different species within the genus. For example, In *L. caucasia*, males have 115–188 and females 119–174 scales around middle of body, respectively. These scales numbers are 80–114 in *L. erythrogaster*, 177–235 in males and 190–259 in female of *L. microlepis microlepis* (Anderson, 1999). In *L. microlepis taftanica* ssp. nov., males have 184–198 and females have 182–200 scales around middle of body (Table 3).

In *L. caucasia* mid-dorsal scales are flattened, keeled and in 7–11 longitudinal rows. In *L. erythrogaster* those scales often are large, keeled and mucronate, in 9–12 longitudinal rows (Rastegar-Pouyani and Nilson, 2002). In *L. microlepis microlepis* mid-dorsal scales are either flat or weakly carinated (Baig et al., 2012). In *L. microlepis taftanica* ssp. nov., mid-dorsal scales are large, weakly

Table 3 Descriptive statistics (mean, standard error, and range) of metric and meristic characteristics of *Laudakia microlepis*, *L. caucasia*, *L. erythrogaster* and *L. microlepis taftanica* ssp. nov.

Characters		SVL	TL	HL	HW	HH	SQ	LVS	AGFS	FCWS
<i>L. microlepis</i> (Female, <i>n</i> = 5)	Mean ± standard error	133.08 ± 3.6	149.35 ± 6.7	32.17 ± 1.9	26.85 ± 1.6	17.32 ± 0.8	210.6 ± 0.9	11.4 ± 0.2	113.8 ± 1.24	28.6 ± 0.67
	range	123.6–145.10	130.75–170.90	27.64–38.22	21.82–30.58	14.83–19.68	208–213	11–12	111–118	27–31
<i>L. microlepis taftanica</i> ssp. nov. (Male, <i>n</i> = 8)	Mean ± standard error	120.1 ± 2.9	161.8 ± 5.2	30.2 ± 0.8	23.6 ± 0.7	14.7 ± 0.6	192.5 ± 1.9	11.9 ± 0.3	124.9 ± 2.1	28.6 ± 0.6
	range	101.6–129.6	127.8–172.1	26.8–32.1	20.1–25.4	10.8–16.1	184–198	11–13	110–136	27–31
<i>L. microlepis taftanica</i> ssp. nov. (Female, <i>n</i> = 6)	Mean ± standard error	114.2 ± 3.1	146.6 ± 7.4	26.4 ± 0.9	21.6 ± 0.7	13.1 ± 0.5	192 ± 2.8	11.3 ± 0.4	123.5 ± 2.5	27.2 ± 1.6
	range	99.7–119.8	121.9–162.8	22.7–29.3	19.8–23.7	11.4–14.1	182–200	10–13	118–130	23–33
<i>L. caucasia</i> (<i>n</i> = 89) ^a	Mean ± standard error	120.1 ± 2.2	170.1 ± 2.7	33.4 ± 0.7	26.5 ± 0.7	15.3 ± 0.5	160.0 ± 3.7	8.4 ± 0.2	102.8 ± 1.6	27.7 ± 0.8
	range	101–140.5	144.1–189.9	27–40	21.9–32.9	12–18.7	123–179	7–10	84–113	25–31
<i>L. erythrogaster</i> (<i>n</i> = 4) ^a	Mean ± standard error	119.9 ± 4.6	152.5 ± 7.6	34.5 ± 1.0	26.8 ± 1.0	16.4 ± 0.9	109.0 ± 6.4	9.5 ± 0.3	88.3 ± 1.1	28.1 ± 0.6
	range	111.0–130.0	139.0–169.0	25.3–38.9	23.1–29.2	15.5–18.9	100–127	9–10	81–94	27–29

^a Measurements adopted from Rastegar-Pouyani and Nilson (2002).

keeled, and arranged in 10–13 oblique longitudinal rows. Also, while *L. caucasia* has enlarged scales on flanks and smooth gular scales (Anderson, 1999), *L. erythrogaster* has flanks with several enlarged mucronate scales and strongly keeled gular scales (Baig *et al.*, 2012), and *L. microlepis microlepis* has flanks with enlarged mucronate scales and small gular scales. *L. microlepis taftanica* ssp. nov., on the other hand, has flanks without enlarged scales and smooth gular scales. Sizes of different *Laudakia* species occurring in Iran are different. The snout-vent length of the largest *L. caucasia* male and female are 153 mm and 152 mm, respectively, and measures 133 mm and 149 mm for the largest male and female of *L. microlepis microlepis* (Anderson, 1999) and 148.5 mm and 150.5 mm for *L. erythrogaster* male and female, respectively (Aghili *et al.*, 2010). Specimens of *L. microlepis taftanica* ssp. nov., were generally smaller than specimens of other species and unlike most other species, in *L. microlepis taftanica* ssp. nov., males were relatively larger than females (129.6 mm in male, 119.8 mm in female) (Table 3).

According to Baig *et al.* (2012) *L. caucasia* has 22–32 scales in the first complete whorl around the tail, whereas *L. erythrogaster* has 24–29 scales. In *L. microlepis taftanica* ssp. nov., those numbers were different for both males and females (27–31 in males and 23–33 in females).

Considering the color patterns, *L. caucasia* is light olive to dark gray, *L. erythrogaster* is olive-brown with many irregular black-edged light marks on vertebral region, and in *L. microlepis microlepis* shoulders and thorax are black, while the head, abdomen and anterior part of tail are light cream to yellow (Anderson, 1999). Even though *L. microlepis microlepis* color pattern is highly variable in different population (Baig *et al.*, 2012), but the color pattern of *L. microlepis taftanica* ssp. nov., is distinct from both the nominal species and other close relative species.

In Iran, *L. caucasia* habitats range from sea level to elevation of about 4 000 m, on the northern parts of the Iranian plateau, while *L. erythrogaster* occupies northeastern parts of Iran to the vicinity of Mashhad, and *L. m. microlepis* habitats are the mountainous areas of the southern, central and eastern parts of the country (Anderson, 1999). *L. microlepis taftanica* ssp. nov. seems to be restricted to the Taftan Mountain at elevation of about 2 500 m in Sistan and Baluchistan province, southeastern part of Iran. It seems that the populations of the new subspecies were once connected to the populations of the nominal species on the most

southeastern edge of their distribution range, when the weather was more suitable and there were connecting populations in between during the last ice age. During the past 10 000 years, however, due to extreme changes in the weather and precipitation patterns (Lomolino *et al.*, 2010) of the area, population of *L. microlepis taftanica* ssp. nov. is separated from populations of *L. m. microlepis* and driven to isolation on higher elevations of Taftan Mountain, where they found refugia, and became distinct in both molecular and morphological traits.

Habitat Typical habitat of this subspecies (Figure 3D) is mountainous terrain, with rocky out crops having crevices. The vegetation coverage consists of sparse *Achillia*, *Astragalus*, *Alhagi*, *Cynodon*, *Phragmites*, *Lilium*, *Tamarix*, *Ziziphora*, *Rumex*, *Artemisia*, *Rheum*, *Zygophyllum*, *Anemone*, *Convolvulus*, *Ephedra* and trees such as *Tamarix*, *Armenica*, *Amygdalus*, *Acer*, and *Morus*.

Distribution *Laudakia microlepis taftanica* ssp. nov., is restricted to the Taftan Mountain at elevations of about 2 500 m, in Sistan and Baluchistan province, southeastern Iran (28° 36' 25.1" N; 61° 04' 47.3" E).

Etymology The new subspecies name is derived from its type locality, Taftan Mountain.

Comparison between different characteristics of lizard specimens sampled from Taftan Mountain, in Iran, with those of close relatives showed that these specimens belong to a new subspecies of *Laudakia microlepis* (Squamata: Agamidae). Reminding the place where this subspecies is found it is called *Laudakia microlepis taftanica* ssp. nov.

Acknowledgements We thank Mr A. Roohi Aminjan for his help with the statistical analyses. We would like to extend our gratitude to Mr H. Parsa and Mr H. Salehi for their assistance in specimen collections.

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